

## REMARKS

Entry of this Amendment and reconsideration of the subject application in view thereof are respectfully requested.

### *I. Claim Status*

Claims 1, 4-24 were pending in the application. Of these, claims 9-24 were withdrawn, and claims 1 and 4-8 were rejected.

### *II. Response to the Office Action*

#### A. Claim Rejections Under 35 USC § 102

The Examiner maintained the rejection of claims 1, 4, 5, 7 and 8 under 35 U.S.C. § 102(b) as being anticipated by Toby, 2001, Methods, 24:201-217 ("Toby"). Applicant respectfully traverses this rejection.

Toby fails to teach each and every limitation of claim 1, either expressly or inherently, for at least the reasons pointed out in the Applicant's amendment and response of July 28, 2010.

At page 5 of the Office Action, the Examiner makes an unsupported assertion that:

The prey comprising nuclear localization signal, B42 activation domain, and HA tag as fusion to inserted cDNA is considered to be a tagged polypeptide, it comprises interacting residue of interaction partner (the cDNA that potentially interacts with the bait), wherein the prey is considered to be a tagged interaction partner of the bait because it binds to the regulatory biomolecule and modulates the activity of the bait."

Applicant respectfully submits that this assertion is misplaced. For example, the limitation, "modulating the activity of a regulatory molecule," set forth in claim 1 is not found, either expressly or inherently described, in the Toby reference. This term refers to the regulation of the nucleic acid binding activity of a biomolecule. See specification at page 6, lines 18-21 (or page 6, 2<sup>nd</sup> full paragraph, 2<sup>nd</sup> sentence). The Examiner cannot establish anticipation by simple mischaracterization of the cited reference. Tobey teaches that the bait remains bound to the DNA whether the DNA is transcriptionally repressed or active. The interaction between bait and

prey causes the transcriptional activation of the reporter gene (e.g., lacZ) but that interaction must be such that it must not affect the nucleic acid binding activity of the bait or biomolecule. Thus, in Toby, the bait protein remains bound to the DNA before, during and after interaction with the prey protein. See Toby generally including Fig. 1. The prey protein does not modulate the activity of the bait. In fact, it is this absolute lack of any modulation in the bait's activity that is not only just essential for, but also the fundamental basis of the functionality of the yeast two-hybrid system described in Toby. Expression of the reporter gene (LacZ) is initiated solely by the B42 activation domain that is part of the interacting protein. The interacting protein's tethering to the unmodulated/unaltered bait protein that remains bound to the DNA leads to the activation of reporter gene expression.

In contrast, in the regulatory system claimed in the present application, the interacting prey protein binds to e.g. Tet repressor and induces an allosteric conformational change of said Tet repressor that leads to the dissociation of TetR from its binding site. This release of TetR from the DNA exemplifies a fundamental aspect necessary for functionality of the claimed application.

Further, for example, the limitations set forth in claim 1(a)(ii) is not found, either expressly or inherently described, in the Toby reference. Specifically, Toby does not teach or disclose "the tag comprises the interacting residues of the interaction partner," and "binds to and modulates activity of said regulatory molecule." In Toby, the interaction with the biomolecule occurs via the bound prey peptide (the variable part of the interaction protein), and not via the tag. In the present application, the tag is the short peptide that interacts with e.g. the TetR protein and which modulates its activity. The tag is the constant part of the interaction protein, the variable part being the cDNA/coding sequence of any gene of interest in the genome of an organism. In the claimed method, the tag is specifically designed (to contain interacting residues) to interact with a regulatory biomolecule, e.g. TetR. It can be added to the coding sequence of any protein to detect the protein expression. Expression of this tagged protein is mediated by the endogenous genetic locus of the gene that was tagged. The "HA tag" (hemagglutinin epitope tag) referred to by the Examiner in the Office Action at, for example, pages 3 and 5, is also a constant part of the molecule described in Toby but this element does not mediate an interaction with the bait protein. One skilled in the art

would know that this element is there to allow one to detect the expression of the interaction proteins (preys) by Western blotting with antihemagglutinin antibody.

These differences clearly represent structural differences between Applicant's claimed system and Toby's system. The yeast two-hybrid system as discussed in Toby requires constant binding of the bait to the DNA to allow reporter gene readout. The method claimed in the present application requires the exact opposite, namely the regulation of the nucleic acid binding activity of the bait protein (release of the "bait" protein, for example, TetR from the DNA) to allow reporter gene readout.

Furthermore, for example, the limitations set forth in claim 1(b) is not found, either expressly or inherently described, in the Toby reference. Specifically, Toby does not disclose or teach the claim requirement that "assessing the expression level of the gene encoding the (poly)peptide of step (a)(ii) via a readout system." The Examiner at page 3 of the Office Action asserts that "the reporter readout reflects the expression [a qualitative feature] of the interacting protein (the prey)." First, the Examiner appears to acknowledge that Toby is not sufficient to meet the specific claim requirement of "assessing the expression *level* [a quantitative parameter] of the gene encoding the (poly)peptide of step (a)(ii) via a readout system." Second, Toby at page 208, in the paragraph bridging left and right columns, states that:

"... the expression of the prey in addition to that of the bait should also be assayed before proceeding to the interaction test. ... Expression of the preys can be assessed by Western blotting with antihaemagglutinin antibody to detect preys expressed in pJG4-5."

This clearly shows that the named authors in Toby do not consider LacZ activity as a method to monitor the expression level of the interaction protein. Toby expressly teaches the use of Western blotting to detect preys, much less expression level of preys. Toby does not teach the use of a reporter protein to detect preys, much less "assessing the expression level preys." One skilled in the art would know that in the systems disclosed in Toby, gene expression is held constant and the interaction itself is variable. This is reinforced in the following sentence at page 208, the last sentence of the paragraph bridging left and right columns:

"If quantitative ranking of the interactions is required, this can be accomplished by performing a liquid ONPG cleavage assay to measure  $\beta$ -galactosidase activity ..."

Thus, the LacZ readout assay is used in Toby to quantify the strength of an interaction between bait and prey (regardless of different baits and preys or of different preys to a single bait), but not at all to assess the expression level of the prey protein as required by the instant claim. See also Toby at page 216, last paragraph, where it teaches:

"In conclusion, although powerful, the two hybrid system is only one of a battery of different techniques that allow detection and refined measurement of protein-protein interactions . . ."

Further, the use of a galactose-dependent inducible promoter referred to in Toby to express the interaction proteins requires the addition of galactose to the growth medium so that the expression of the prey is obtained. In sum, the concept of using reporter readout to assess the expression level of the prey is not taught or disclosed in Toby. Accordingly, Toby fails to teach each and every limitation of claim 1, either expressly or inherently, which teaching is required for the reference to anticipate the claimed invention.

To anticipate, the cited reference must also enable one of ordinary skill in the art to make and use the claimed invention. The presently claimed method uses a tag specifically designed to interact with e.g. TetR that can be easily added to the coding sequence of any protein to detect its expression. As discussed above, Toby is nothing to do with "assessing the expression level of the gene encoding the (poly)peptide of step (a)(ii) via a readout system." There is no teaching in Toby to enable one skilled in the art to carry out the claimed method. Further, Applicant respectfully submits that the yeast two-hybrid system of Toby is not functional in bacteria. Activation of gene expression is mediated mainly by alteration in DNA binding activity of entire repressor or activator proteins. An activation domain like the B42 domain interacts specifically with protein cofactors of eukaryotic RNA polymerase II, which are not present in bacteria, as these use a completely different mechanism to initiate transcription. This constitutes a clear limitation of use of a yeast two-hybrid-like system to monitor gene expression. Toby is not an enabling prior art reference.

Accordingly, Toby fails to anticipate claim 1. Claims 4-8, at least by virtue of its dependency, are similarly considered by the Applicant to patentably define themselves and are novel over Toby. Reconsideration and withdrawal of this rejection are respectfully requested.

B. Claim Rejections Under 35 U.S.C. § 103

The Examiner maintained the rejection of claim 6 under 35 U.S.C. § 103(a) as being obvious over Toby, 2001, Methods, 24:201-217 ("Toby") in view of Manfredi et al., U.S. Patent 6,828,112 ("Manfredi"). This rejection is respectfully traversed and believed to be overcome in view of the following discussion:

Claim 6, which depends from claim 1, requires a protein that confers resistance to an antibiotic.

The Examiner acknowledges that Toby does not teach using a protein conferring antibiotic resistance as the reporter protein. However, the Examiner appears to be arguing that Manfredi cures the deficiencies in Toby.

Toby is discussed above. Toby focuses on the yeast-two-hybrid systems. For at least the reasons discussed above, Toby does not teach or suggest a method for monitoring the expression level of a gene in a host cell by modulating the activity of a regulatory biomolecule. Like Toby, Manfredi discloses yeast-two-hybrid systems. Manfredi does not remedy deficiencies in Toby. Specifically, for example, Manfredi does not teach or suggest "modulating the activity of a regulatory molecule." Manfredi does not teach or suggest that "the tag comprises the interacting residues of the interaction partner," and "the interaction partner binds to and modulates activity of said regulatory molecule." Manfredi does not teach or suggest anything about "assessing the expression level of the gene encoding the (poly)peptide of step (a)(ii) via a readout system." The Examiner has not explained why Manfredi's teachings about a method for detecting "protein-protein interactions" could have prompted one of ordinary skill in the art to modify Toby in a predictable manner to arrive at the claimed invention. The Examiner has not identified a reason that would have prompted one of ordinary skill in the art in the field to transform a cell expressing a regulatory biomolecule with a nucleic acid molecule comprising a tag with the interacting residues of the interaction partner that binds to and modulates activity of said regulatory biomolecule such that the expression level of the gene encoding the (poly)peptide of step (a) (ii) via a readout system provided by a reporter protein can be assessed



In view of the foregoing, Applicant respectfully submits that the Examiner has not established a *prima facie* case of obviousness of claim 6 or other pending claims, under 35 U.S.C. § 103(a). Accordingly, reconsideration and withdrawal of this rejection are respectfully requested.

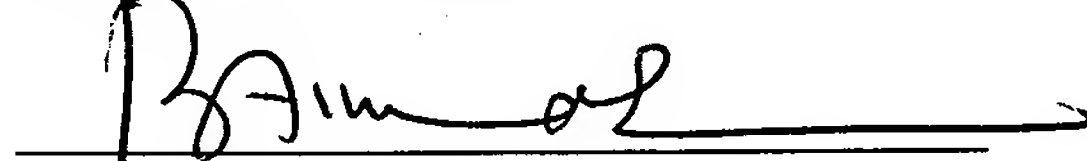
### **III. Conclusion**

Applicant believes this submission to be a full and complete response to the Office Action. Accordingly, favorable reconsideration in view of this response and allowance of all of the pending claims are earnestly solicited.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the present application, the Examiner is invited to call the undersigned attorney.

November 7, 2011

Respectfully submitted,  
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